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Persistent Epstein–Barr virus infection in a human T-cell line: unique program of latent virus expression

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The growth transforming potential of Epstein–Barr virus (EBV) for Burkitt's lymphoma and nasopharyngeal carcinoma is now extended to other neoplasia, such as Hodgkin's disease, peripheral T-cell tumor and gastric cancer. We have generated an EBV recombinant with a selectable marker at the viral thymidine kinase locus. Recombinant EBV was successfully infected into a human T-cell line, MT-2. Following incubation in the selective medium, drug resistant MT-2 cell clones were isolated and proved to be infected with recombinant EBV. EBV-infected MT-2 cell clones expressed EBNA 1 and LMP 1 and very little of EBNA 2, showing the *Bam*HI F promoter-driven latency II form of infection, which is seen in non-B-cell tumors. This is the first report of *in vitro* generation of latency II type EBV infection. The present system of persistent EBV infection in T cells should be a good model for investigating the pathogenic role of EBV in non-B-cell tumors.

Keywords: *Bam*HI F promoter/CD21/Epstein–Barr virus/recombinant virus/T cell

Introduction

Epstein–Barr virus (EBV) infection occurs in oropharyngeal epithelial cells with subsequent spread of the virus to peripheral lymphocytes. The major target cells of EBV *in vivo* are B lymphocytes and squamous epithelium. The oncogenic potential of EBV has been indicated in Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC). Recently, EBV genomes have been detected in other neoplasia, such as peripheral T-cell lymphoma (Harabuchi *et al.*, 1990), Hodgkin's disease (Weiss *et al.*, 1989) and gastric cancer (Shibata *et al.*, 1991).

The EBV infects B lymphocytes through CD21. EBV binding to CD21 is mediated by the viral envelope protein, gp350/220. In addition to B lymphocytes, CD21 is expressed on certain leukemic T-cell lines, immature thymic T cells, and normal peripheral blood T cells (Tsoukas and Lambris, 1993). Several reports mention the infection by EBV of cells of the T-cell lineage (Warty *et al.*, 1991; Koizumi *et al.*, 1992; Sinha *et al.*, 1993). These studies only showed transient EBV expression in part of the cell cultures. So far, very little is known about the interaction between EBV and T-cell tumors.

The expression of episomal EBV genes during latency establishes transformation and immortalization of B lymphocytes. Three distinct forms of EBV latent gene expression have been described (Rowe *et al.*, 1992). B lymphoblastoid cell lines (LCL) transformed by EBV *in vitro* display the full pattern of latent gene expression (latency III) encompassing six nuclear antigens (EBNAs 1, 2, 3A, 3B, 3C and leader protein) and three latent membrane proteins (LMPs 1, 2A and 2B). In latency III infection, EBNA mRNAs are differentially spliced from long transcripts initiated from either *Bam*HI C promoter (Cp) or downstream *Bam*HI W promoter (Wp), while the LMP transcripts are expressed from separate promoters in the *Bam*HI N region that are responsive to transactivation by EBNA 2. EBV-positive BL cells express only EBNA 1 and no other viral proteins (latency I). EBNA mRNA transcription is driven by *Bam*HI F promoter (Fp). In contrast to the well-characterized forms of latency mentioned above, latency II which expresses all the LMPs and Fp-driven EBNA 1 was scarcely recognized in B cells. This type of latent infection was recognized in non-B-cell tumors, such as NPC (Brooks *et al.*, 1993), T-cell tumors (Minarovits *et al.*, 1994) and Hodgkin's disease (Deacon *et al.*, 1993). In latency II infection, LMP can be expressed independently of EBNA 2-mediated transactivation. Thus far, there has been no *in vitro* model of latency II infection.

In this paper, generation of an *in vitro* system, which allows persistent infection of EBV in T-cell lines is described. A human T-lymphotropic virus type I (HTLV-I)-infected T-cell line, MT-2 (Miyoshi *et al.*, 1981) was infected with recombinant EBV with a selectable marker, the G418 resistance gene (*neo*^{r}). We show that recombinant EBV can persistently infect MT-2 cells with expression of EBNA 1 and LMP 1 and very little of EBNA 2, showing the unique latency II form of EBV infection.

Results

Generation of an EBV recombinant with a selectable marker at the viral thymidine kinase (TK) locus

Akata cells (Takada, 1984; Takada and Ono, 1989) have ~20 copies of EBV plasmid per cell. Akata cells have a unique property in that they start to produce EBV after cross-linking of cell surface immunoglobulins (Ig) with anti-Ig antibodies. The *neo*^{r} gene was inserted into an EBV plasmid of Akata cells, by using homologous recombination. The pXneo plasmid contained the *neo*^{r} gene under control of the SV40 early promoter replaced BXLF-1, an open reading frame encoding the TK gene, which is a homologue of the herpes simplex virus type 1 and is non-essential for infection and replication (Figure 1A). The surrounding EBV DNA could target the marker into the EBV genome in a reading frame expected to be non-essential. The plasmid was transfected into Akata cells,

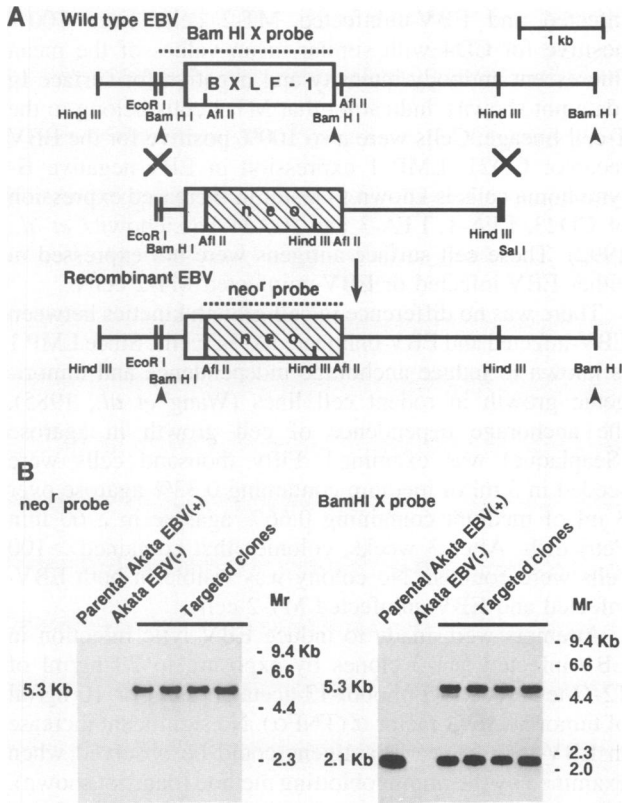

Fig. 1. Generation of recombinant EBV with a selectable marker. (**A**) Schematic representation of plasmid constructs used to insert *neo*^{r} gene into the EBV genome. Map of the BXLF-1 region is shown at the top (Baer *et al.*, 1984). The pXneo plasmid at the second line was derived by inserting an SV40 early promoter-driven *neo*^{r} gene into the BXLF-1 open reading frame in an EBV (Akata) *Eco*RI–*Hin*dIII DNA clone. When the pXneo plasmid had recombined into the BXLF-1 site, a novel 5.3 kb *Bam*HI fragment should appear due to a deletion of *Bam*HI site on the right (the bottom line). (**B**) Southern blot analysis of targeted Akata cell clones. Ten μg of cellular DNAs were digested with *Bam*HI, blotted, and hybridized with the *neo*^{r} or *Bam*HI X probe.

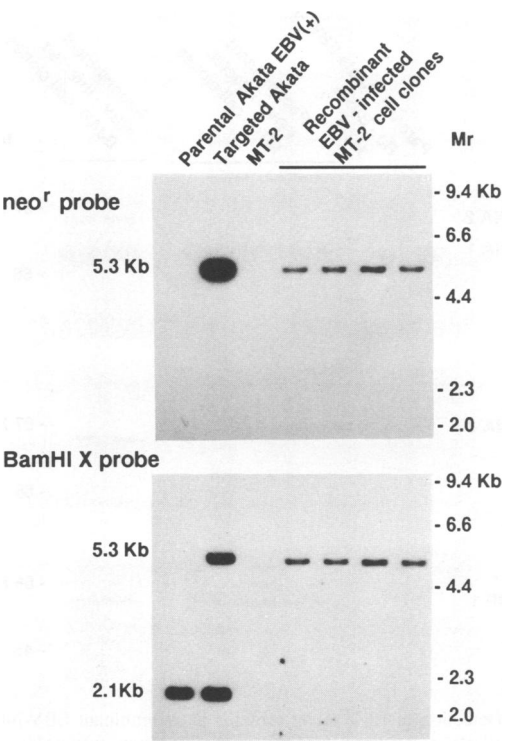

Fig. 2. Southern blot analysis of recombinant EBV-infected MT-2 cell clones. Ten micrograms of cellular DNAs were digested with *Bam*HI restriction enzyme, blotted, and hybridized with ^{32}P-labeled *neo*^{r} or *Bam*HI X probe.

and Akata cell clones resistant to G418 were selected. Six hundred and seven G418-resistant Akata cell clones appeared from a total of 3840 wells tested. Viruses released into the supernatant of anti-Ig-treated cultures were analyzed by Southern blotting. Four out of 607 resistant clones proved to contain recombinant EBV. Figure 1B shows four clones that contained EBV recombinants with the *neo*^{r} DNA recombined into the expected EBV BXLF-1 site, as well as non-recombinant Akata EBV. This was evidenced by the presence of the 2.1 kb *Bam*HI X fragment and the concurrent appearance of a novel 5.3 kb *Bam*HI fragment which hybridized to both *Bam*HI X and *neo*^{r} probes.

EBV infection of MT-2 cells

MT-2 cells were infected with culture supernatant from G418-resistant Akata cells, which contained a mixture of wild-type and recombinant EBV. After 3 weeks incubation in the selective media, 30 G418-resistant clones appeared from a total of 576 wells tested. Ten clones were analyzed to ascertain whether cells were infected with EBV. These clones all proved to be infected with only recombinant EBV. Examples of results showing evidence for infection with only a recombinant virus and not with a parental Akata virus are given in Figure 2. Southern analysis indicated that the EBV DNA in MT-2 cells has the *neo*^{r} DNA exactly recombined into the BXLF-1 site. This was evidenced by the absence of the 2.1 kb *Bam*HI X fragment and the presence of a 5.3 kb *Bam*HI DNA fragment which hybridized to both *Bam*HI X and *neo*^{r} probes (Figure 2).

EBV-infected MT-2 cell clones were virtually 100% positive for EBNA by anti-complement immunofluorescence with a polyvalent human antiserum. Further analysis of EBV-infected MT-2 cell clones by immunoblot analysis demonstrated that all cell clones were positive for EBNA 1 and LMP 1, but negative for EBNA 2 (Figure 3). This result was in contrast to the pattern of EBV gene expression in BJAB cell clones that were infected with the same recombinant EBV preparation, in which strong EBNA 2 expression was observed. The pattern of EBNA 1 promoter usage in EBV-infected MT-2 cell clones was examined by RT-PCR analysis. The results indicate that EBV-infected MT-2 cell clones utilize the *Bam*HI F promoter exclusively, and not *Bam*HI C/W promoters, to transcribe the EBNA 1 gene (Figure 4). The expression of LMP 2A, LMP 2B and *Bam*HI A antisense transcripts was also examined by RT-PCR analysis. As shown in Figure 5, recombinant EBV-infected MT-2 cell clones expressed all of these transcripts. Their patterns of viral gene expression are those typically observed in latency II cells.

Further analysis of these clones using the system of Gardella *et al.* (1984) demonstrated that recombinant EBV genomes were maintained as episomes in MT-2 cells (Figure 6).

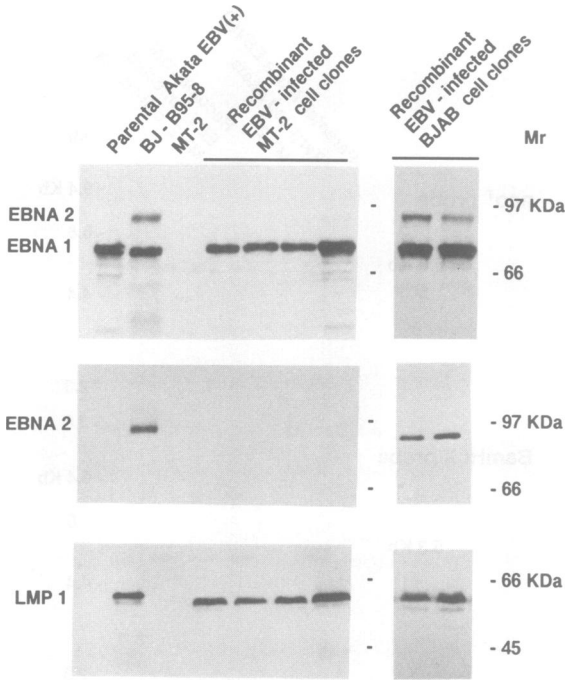

Fig. 3. Detection of EBV latent proteins in recombinant EBV-infected MT-2 cell clones by Immunoblot analysis. For immunostaining, the top blot was treated with a standard EBNA-positive human serum and peroxidase-labeled protein A. The second blot was treated with an EBNA 2 monoclonal antibody (PE 2) (Young *et al.*, 1989) and peroxidase-labeled anti-mouse Ig. The bottom blot was treated with an LMP 1 monoclonal antibody (CS1-4) (Rowe *et al.*, 1987) and peroxidase-labeled anti-mouse Ig.

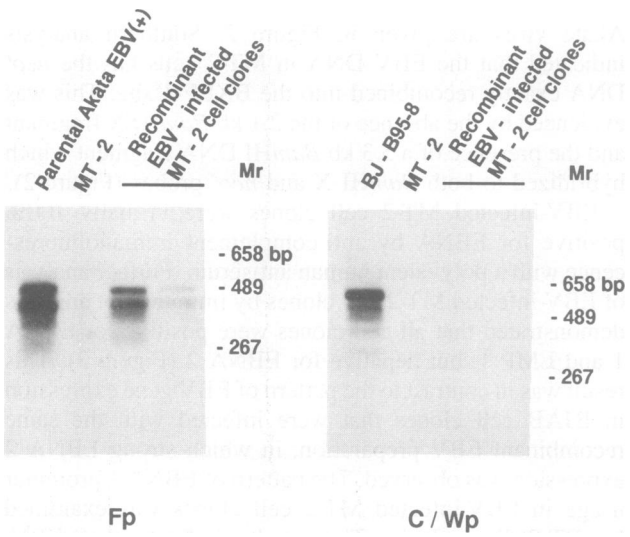

Fig. 4. RT-PCR analysis of EBNA 1 transcripts in EBV-infected MT-2 cell clones. EBNA 1 transcripts originating from the F promoter (Fp) or C/W promoter (C/Wp) region were amplified with primer pairs described in Materials and methods. Southern analysis was performed with an internal oligonucleotide probe capable of hybridizing to transcripts arising from Fp or C/Wp. Akata cells and BJ-B95-8 cells were used as positive controls for Fp- and C/Wp-driven transcripts, respectively.

Characteristics of EBV-infected MT-2 cells

Cell surface antigen expression in EBV-infected and EBV-uninfected MT-2 cells was examined by fluorescence-activated cell sorting analysis (Figure 7). Both EBV-infected and EBV-uninfected MT-2 cells were 100% positive for CD4 with similar mean values of the mean fluorescent antibody intensity and negative for surface Ig (data not shown), indicating that MT-2 cells belong to the T-cell lineage. Cells were also 100% positive for the EBV receptor CD21. LMP 1 expression in EBV-negative B-lymphoma cells is known to result in increased expression of CD23, LFA-1, LFA-3 and ICAM-1 (Liebowitz *et al.*, 1992). These cell surface antigens were not expressed in either EBV-infected or EBV-uninfected MT-2 cells.

There was no difference in cell growth kinetics between EBV-infected and EBV-uninfected MT-2 cells. Since LMP 1 is known to induce anchorage independence and tumorigenic growth in rodent cell lines (Wang *et al.*, 1985), the anchorage dependence of cell growth in agarose (Seaplaque) was examined. Fifty thousand cells were seeded in 3 ml of medium containing 0.33% agarose over 3 ml of medium containing 0.66% agarose in a 60 mm Petri dish. After 3 weeks, colonies that contained >100 cells were counted. No colony was visible in both EBV-infected and EBV-uninfected MT-2 cells.

Attempts were made to induce EBV lytic infection in EBV-infected MT-2 clones by exposure to 20 ng/ml of 12-*O*-tetradecanoyl-phobol-13-acetate (TPA) or 10 ng/ml of tumor necrosis factor α (TNFα). No significant increase in EBV-specific, early antigens could be observed when examined by the immunoblotting method (data not shown).

HTLV-I expression in EBV-infected MT-2 cells

MT-2 cells spontaneously produce a large amount of HTLV-I. We examined whether EBV infection had any effect on HTLV-I production in MT-2 cells. As shown in Figure 8, there was no difference in the intensity of HTLV-I polypeptides (Ohtsu *et al.*, 1987). TPA or TNFα treatment had no effect on the intensity of HTLV-I polypeptides.

Discussion

In the present study, an EBV recombinant with a selectable marker was generated and successfully infected into a human T-cell line, MT-2. Following incubation in the selective media, many drug-resistant MT-2 cell clones were isolated very easily and proved to be infected with recombinant EBV. Our results show that under certain circumstances, T lymphocytes express CD21 and are susceptible to infection with EBV, which may initiate the development of EBV-positive T-cell tumors. A high level expression of CD21 may explain an efficient EBV infection in MT-2 cells. However, we failed to infect other T cells, such as Molt-4, MT-4 and peripheral T lymphocytes, with EBV. Whether this is due to insufficient receptor density, microviscosity of the cellular membrane lipids (Patel *et al.* 1993), or other as yet unidentified factors remains to be clarified.

EBV-infected MT-2 cell clones express EBNA 1 and LMP 1 and very little EBNA 2. Gardella gel analysis demonstrated that recombinant EBV genomes were maintained as episomes in MT-2 cells. Furthermore, when the same recombinant EBV was used to infect BJAB cells, strong EBNA 2 expression was observed. These results indicate that the recombinant EBV contains the intact EBNA 2 gene and exclude the possibility that EBNA 2 expression in MT-2 cells was interrupted by the integration

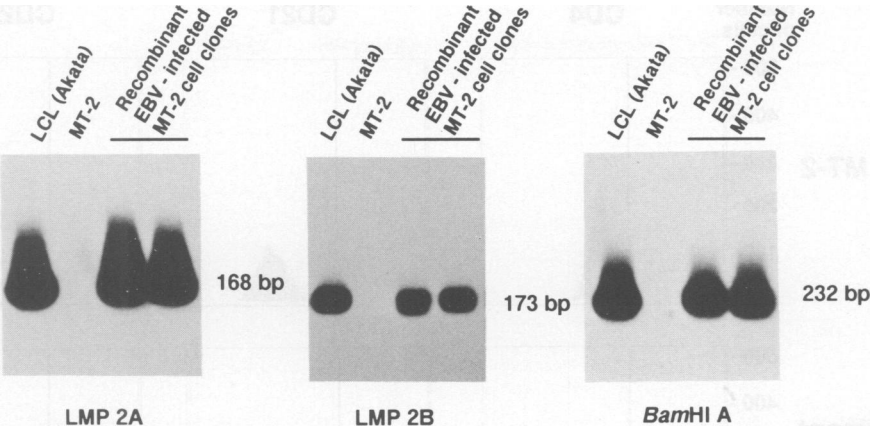

Fig. 5. RT-PCR analysis of LMP 2A, LMP 2B and *Bam*HI A transcripts in EBV-infected MT-2 cell clones. LCL (Akata) is an Akata EBV-transformed lymphoblastoid cell line used as a positive control for these transcripts.

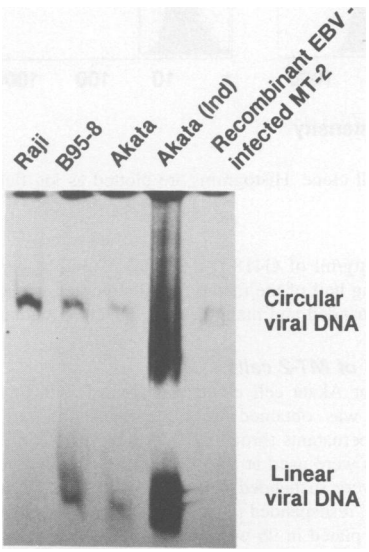

Fig. 6. Gardella gel analysis of recombinant EBV-infected MT-2 cell clones. Raji cell contains stable copy number of EBV genomes (~45 genome equivalents per cell) in a closed circular form. B95-8 cells contained both circular and replicating linear viral DNAs. Linear viral DNA in Akata cells reflected spontaneous EBV replication, in which amplification of linear viral DNAs was induced after anti-Ig treatment.

of EBV DNA into cellular DNA. Therefore, it is unlikely that EBNA 2 expression in MT-2 cells was interrupted by the integration of EBV DNA into cellular DNA. RT-PCR analysis showed that in MT-2 cells, the *Bam*HI F promoter is used for expressing EBNA 1. This was characteristic of the latency II form of EBV infection. This type of latent infection was recognized only in non-B-cell tumors, such as NPC (Brooks *et al.*, 1993), T-cell tumors (Minarovits *et al.*, 1994) and Hodgkin's disease (Deacon *et al.*, 1993). The latency II infection has not been well characterized, because of the lack of an *in vitro* model for latency II infection. This is the first report of *in vitro* generation of latency II type EBV infection. This model should facilitate the investigation of the pathogenic role of EBV in non-B-cell tumors.

EBV, with a selectable marker, is very useful for selecting EBV infected cells, especially when the efficiency of infection is low or the EBV-uninfected population in cultures are able to proliferate. The recombinant EBV was successfully infected into EBV-negative Akata cells and amplified in those cells via induction of their lytic cycle with anti-Ig, which made it possible to produce a large amount of recombinant EBV (H.Yoshiyama, N.Shimizu and K.Takada, manuscript in preparation). Transplanting the EBV receptor (CD21) makes it possible to overcome the barrier of virus adsorption to the target cells. EBV infection in various cell types may elucidate cellular factors which determine viral gene expression. We are now investigating whether IL-2 dependent peripheral T lymphocytes become IL-2 independent after EBV infection following CD21 transfection. This experiment will provide an answer as to whether EBV transforms T lymphocytes.

LMP 1 expression in MT-2 cells did not change growth characteristics. This is conceivable, since growth promoting effects of LMP 1 were only shown in some rodent cell lines (Wang *et al.*, 1985). LMP 1 expression in MT-2 cell did not alter the expression of CD23, LFA-1, LFA-3 or ICAM-1, which were known to be induced by LMP 1 in B-lymphoma cells (Liebowitz *et al.*, 1992). The result suggests that B-cell factors may be required for expression of these cell surface antigens.

Materials and methods

Cell lines, cell culture and induction of virus replication

The Akata cell line (Takada, 1984; Takada and Ono, 1989) is derived from an EBV-positive BL and expresses surface Ig of the G (κ) class. The Akata cell line was subcloned and 100% EBV-positive Akata clones and completely EBV-negative Akata clones were isolated (Shimizu *et al.*, 1994). The MT-2 cell line (Miyoshi *et al.*, 1981) is an HTLV-I producer and is derived from cord blood T lymphocytes that have been co-cultivated with leukemic cells from a patient with adult T-cell leukemia. BJ-B95-8 is generated by infecting EBV (B95-8) with an EBV-genome negative B-lymphoma cell line, BJAB. All cells were maintained in complete culture medium consisting of RPMI 1640 supplemented with 10% fetal bovine serum (Hyclone), penicillin (40 U/ml), streptomycin (280 μg/ml) at 37°C in a 5% humidified CO_2 atmosphere.

To induce EBV production in Akata cells, 5×10^6 cells were incubated in 5 ml of complete medium containing 1% (v/v) goat antibodies specific for human IgG (Cappel) for 48 h (Takada and Ono, 1989). To induce EBV and HTLV-I production in MT-2 cells, 5×10^6 cells were incubated in 5 ml of complete medium containing 20 ng/ml of TPA or 10 ng/ml of TNFα for 3 days.

Construction of the plasmid for generating recombinant EBV with a selectable marker

The 4.3 kb *Eco*RI–*Hin*dIII fragment of Akata EBV DNA, which includes the EBV TK gene encoded by a leftward open reading frame BXLF-1

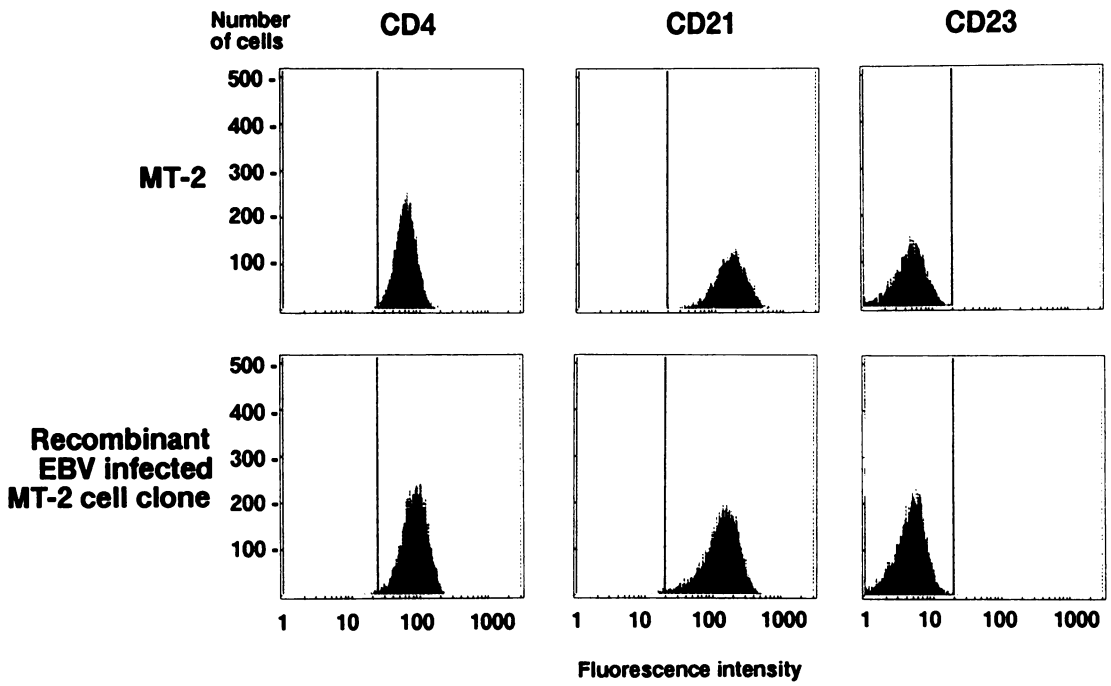

Fig. 7. Cell surface antigen expression in a representative recombinant-EBV infected MT-2 cell clone. Histograms are plotted as log fluorescence intensity (*x*-axis) versus cell number (*y*-axis).

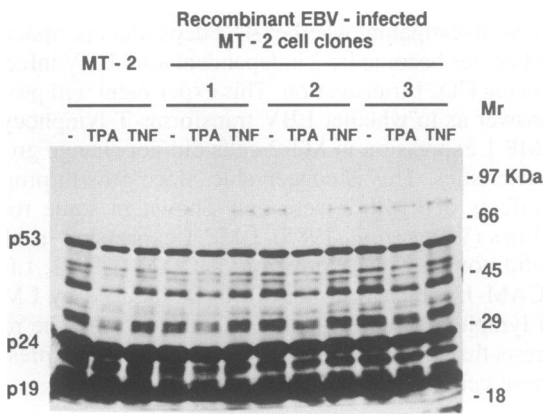

Fig. 8. HTLV-I expression in recombinant EBV-infected MT-2 cell clones. Immunoblot analysis of protein extracts from cells non-treated (–) or treated with 20 ng/ml of TPA or 10 ng/ml of TNFα. For immunostaining, the blot was treated with a standard HTLV-I positive serum from a patient with adult T-cell leukemia and peroxidase-labeled protein A. p53, p24 and p19 are major HTLV-I polypeptides (Ohtsu *et al.*, 1987).

of the *Bam*HI X fragment, was cloned into the multicloning site of pBluescript II KS+ (pBS). The plasmid DNA was digested with *Afl*II and treated with calf intestinal alkaline phosphatase. The 1.7 kb *Afl*II fragment in the BXLF-1 gene was replaced with the 1665 bp *Sal*I–*Eco*RI fragment from pDOL- (Korman *et al.*, 1987) containing the SV40 promotor-driven *neo*^{r} gene. The resultant plasmid was named pXneo.

Generation of recombinant EBV

The pXneo plasmid was transfected into Akata cells by the electroporation method. Cells (5×10^6) were suspended in 500 µl of ice-cold phosphate-buffered saline (PBS) containing 40 µg of pXneo DNA that was doubly digested with *Eco*RI and *Sal*I to release the *neo*^{r} DNA and the surrounding EBV DNA from the pBS vector. Cells were then electroporated with an Electro Cell Manipulator 600 (BTX) at room temperature in cuvettes having a 0.4 cm electrode gap. Transfected Akata cells were cultured in 5 ml of culture medium for 2 days, and then transferred to 96-well, flat-bottom plates at 5000 cells per well in complete culture medium containing 700 µg/ml of G418 (Life Tecq). Cultures were fed every 5 days by replacing half of the medium until colonies emerged (3 weeks). Clones were expanded and maintained in selective medium.

EBV infection of MT-2 cells

A G418-resistant Akata cell clone was treated with anti-Ig for 48 h. Cell-free EBV was obtained by centrifuging induced cultures and filtrating the supernatants through a 0.45 µm filter. For EBV infection, the supernatants were used at a 1:10 dilution in growth medium. MT-2 cells (5×10^6) were suspended in 1 ml of diluted EBV supernatant for 90 min, pelleted, resuspended in fresh medium and incubated for 2 days. Cells were then plated in 96-well, flat-bottom plates at 10 000 cells per well with complete medium containing 500 µg/ml of G418. Cells were fed every 5 days until colonies emerged (2–3 weeks).

Southern blot analysis

Purified cellular DNA (10 µg) was digested with *Bam*HI restriction enzyme, precipitated and resuspended in 10 µl of loading buffer. The DNA was size-fractionated by electrophoresis in a 0.8% agarose gel and transferred to a nylon membrane (Hybond N+; Amersham). Probe DNAs were labeled with [^{32}P]dCTP (3000 Ci/ml) by the random primer-based method (Multiprime DNA labeling system; Amersham). Hybridization was performed in 5% dextran sulfate (mol.wt. 500 000, Sigma), 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 20× dilution of liquid blocking agent (supplied by Amersham) and 10 µg/ml of salmon testis DNA (Sigma) for 13 h at 60°C. The filter was washed in 1× SSC, 0.1% SDS at 60°C for 15 min, then 0.1× SSC, 0.1% SDS at 60°C for 15 min, and was then exposed to X-ray films.

Western blot analysis

Cell pellets were lysed in SDS–PAGE loading buffer. After the pellets were boiled for 5 min, equal amounts of protein (corresponding to 2.5×10^5 cells) were separated in 10% polyacrylamide gels and transferred to nitrocellulose membrane. The blots were blocked with 5% milk in PBS. After immunostaining, the blots were developed by the enhanced chemiluminescence (ECL) method (Amersham) according to the manufacturer's protocol.

RT-PCR analysis

Total RNA was extracted by the acid guanidinium thiocyanate–phenol–chloroform method (Chomczynski and Sacchi, 1987). One µg of extracted RNA was used for cDNA synthesis. Analysis of promoter usage for expression of EBNA 1 was performed as follows. cDNA synthesis was

primed by 3′ PCR primer (5′ TGACGGGTTTCCAAGACTATCC 3′) (Qu and Rowe, 1992) in a reaction mixture consisting of 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 5 mM $MgCl_2$, 1 mM deoxynucleotides, 1 U RNase inhibitor, 2.5 U reverse transcriptase and 16 pmol 3′ PCR primer. The reaction mixture (20 μl) was incubated at 42°C for 15 min followed by inactivation of reverse transcriptase by heating at 99°C for 5 min. For PCR, 16 pmol of 5′ PCR primer (5′ ACCTCCTGTCACCACCTCC 3′ for F promoter, 5′ AGAGGAGGTGGTAAGCGGTTC 3′ for C/W promoter) (Qu and Rowe, 1992) and 2.5 U of *Taq* DNA polymerase were added and the buffer conditions were adjusted to 10 mM Tris–HCl (pH 8.3), 2 mM $MgCl_2$. Reaction mixtures were subjected to 35 cycles of denaturation (94°C, 30 s), annealing (55°C, 30 s) and extension (72°C, 1 min). Ten microliters of PCR products were size-separated on 2% agarose gels, blotted to nylon membranes, and visualized by the ECL 3′-oligolabelling and detection system (Amersham) with antisense oligonucleotide of EBNA 1 coding sequence (5′ GATGTGTCTTCCTT-CTGTCCTA 3′) as a probe.

For detection of latent EBV transcripts, the following primer pairs were used (Qu and Rowe, 1992; Brooks *et al.*, 1993), with the same amplification protocols as described above. LMP 2A (5′ GCAACACG-ACGGGAATGACG 3′ and 5′ AAACACGAGGCGGCAATAGC 3′), and LMP 2B (5′ TGGGAAGCGGCAGTGTAATC 3′ and 5′ AAACA-CGAGGCGGCAATAGC 3′), and *Bam*HI A (5′ AGAGACCAGGCTGC-TAAACA 3′ and 5′ AACCAGCTTTCCTTTCCGAG 3′). Probes used are 5′ GCAACAATTACAGGCAGGCATAC 3′ for LMP 2A and 2B, and 5′ AAGACGTTGGAGGCACGCTG 3′ for*Bam*HI A.

Gardella gel analysis

Analysis of linear and circular viral DNA in recombinant EBV-infected MT-2 cells was carried out by the method of Gardella *et al.* (1984) with modifications (Gardella *et al.*, 1984; Hurley and Thorley-Lawson, 1988). EBV-infected cells were lysed in a well of agarose gel. Cellular DNA and integrated viral DNA are too large to enter the gel, but circular and linear EBV DNA will enter the gel. Differential mobility of circular and linear EBV DNA can be recognized. Horizontal gel of 0.75% agarose was poured, and the area above the well was removed and replaced with 0.8% agarose containing 1% SDS and 1 mg/ml of pronase E. Two million whole cells were placed in each well. Electrophoresis was carried out at 4°C at 20 V for 3 h, then the voltage was increased to 100 V for an additional 13 h. The viral DNAs were alkaline-blotted onto a nylon membrane by using a vacuum blotter (VacuGene XL, Pharmacia LKB). The viral DNAs were hybridized with ^{32}P-labelled EBV *Bam*HI K fragment. After washing, the viral DNAs were detected by autoradiography.

Acknowledgements

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